Temperature acclimation in dragonfly larvae: which species are more vulnerable to global warming?

Erik Karlsson

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Biology Education Centre and Department of ecology and genetics/Animal ecology, Uppsala University
Supervisor: Frank Johansson
External opponent: David Outomuro
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Abstract

Climate change is affecting all known habitats on earth. Increased temperatures in aquatic habitats will not only heat the waters but will also cause larger variation in temperature fluctuation. Many animals in aquatic systems are adapted to specific habitats in these waters and may face disadvantages if temperatures changes too much.

In my project, I have used larvae of dragonflies and damselflies to examine how they might be affected by changes in water temperature caused by climate change. I sampled damselfly and dragonfly larvae in three lakes around Uppsala at two water depths, in order to examine whether these species differed in their depth distribution. Larvae exposed to thermal fluctuations should be better at adapting to the increase in temperature from global warming. In a laboratory experiment I tested the ability to acclimate to three temperatures (18, 21 and 24 °C) in three species of damselfly larvae (Ischnura elegans, Coenagrion pulchellum, and Erythromma najas), over four different time intervals (1, 2, 12 and 24 hours) for E. najas and two time intervals for I. elegans and C. pulchellum. To measure acclimation, I introduced prey items in a laboratory experiment, and counted how many strikes and captures the larvae managed to do during a 10 min interval after being acclimated to the four different time intervals. The results of the field sampling in the lakes showed species specific depth distribution differences in dragonfly and damselfly larvae, with the majority of species preferring the surface over the bottom.

The results from the temperature acclimation experiments showed that species changed their strikes and capture success on prey over the different time intervals, and especially E. najas showed a clear trend that suggest adaptation to temperate changes. Species differed in prey capture behaviour, with E. najas having the highest and C. pulchellum the lowest number of strikes and capture success. Results also suggested that E. najas is able to acclimate to the temperatures over the time period tested. This species also had a wide depth distribution in the lakes, which might explain its faster acclimation, even though a similar depth distribution was found in the non acclimating C. pulchellum. The other two species (besides E. najas) showed less clear patterns with regard to acclimation and it was difficult to tell if they were able to acclimate to the tested temperatures. My results suggest that damselfly larvae species are able to acclimate in prey capture behaviour over relatively short time periods in response to temperature fluctuations, and they might therefore be able to adapt to increasing temperature fluctuations in the future.
Introduction

Anthropogenic climate change is having a significant impact on the ecosystems on earth (IPCC 2013). Due to rising temperatures on our planet we see changes in how well organisms perform in their natural habitats. These changes occur in the Antarctic (Mintenbeck et al. 2012), brackish waters of the Baltic Sea (Kotta et al. 2014) and the rivers of Europe (Floury et al. 2013). Climate change affects organisms differently, while some fare better, others are negatively impacted. Nevertheless, the overall effects are clearly negative to many aquatic animals, including e.g. crocodiles (Rodgers et al. 2015) and stone- and mayfly species (Li et al. 2013, Floury et al. 2013). Biotic interactions are also affected. For example, benthic interaction in Finnish lakes has been shown to change in response to climate change (Jyväsjärvi and Hämäläinen 2015). Also, many terrestrial ecosystems are affected (Angert et al. 2011, Deutsch et al. 2008).

The majority of organisms on the planet are ectotherms and thus have a very low degree of internal temperature generation and have usually more or less the same temperature as their environment, although this can change with for example muscle contractions or activity. Large proportions of these organisms are insects and they are either ectotherms or facultative endotherms (Angiletta 2009), which means that their performance is affected by the temperature in their environment. For example, the metabolism and activity of the organism is associated with the surrounding temperature such that warmer temperature allows for higher metabolism and activity, while cold environments results in lower metabolism and activity.

For organisms living in water, the temperature effect might be more important compared to terrestrial organisms, since water transports energy and temperature effects much stronger than air. Partially water acts as a buffer; however this buffer only accounts to some degree and will change with the increase of temperature on earth. Higher temperature will allow body processes to happen faster. High temperatures will thus allow an animal to have the ability to metabolize faster, but only to a certain degree, before the heat kills them at the critical thermal maximum (CTMax). Hence, too high temperatures will make enzymes degrade and denaturize proteins (Somero 1995). Likewise there is a critical thermal minimum (CTMin), which is the temperature at the low end that kills animals. The concept of CTMax and CTMin is useful for determining the limits of temperature adaption (Cowles and Bogert 1944).

One of the challenges for predicting how organisms react to changes in temperature increase, for example due to global warming, is that organisms can adapt physiologically to
temperature changes. This thermal adaption is termed acclimation and is usually defined as any phenotypic response to environmental temperature that alters performance and plausibly enhances fitness (Angilletta 2009). Angilletta (2009) defines two types of acclimation: developmental and reversible. Developmental acclimation is a set response to a certain environment, which is changed during development. Reversible acclimation is regulated by variation in change in temperature during an individual’s lifetime. However, as Angilletta (2009) points out, we cannot always with certainty distinguish between the two of them in practice. Nevertheless, both these types of acclimation require the detection of environmentally transmitted signals that trigger a response in the cells of the animal and change its phenotype when activated (Angilletta 2009). Acclimation is however not without costs. It may even be a disadvantage for the animal. For example, producing energetically and metabolically expensive heat shock proteins when low on resources might be costly (Li and Srivastava 2004).

Since temperature changes at a seasonal scale and also at short time intervals such as day and night, it is interesting to examine how animals have adapted and differ in their adaptation to these fluctuations in temperature. Further, as current global warming might affect these fluctuations it is important to understand such thermal adaptations. Thus, animals living in aquatic ecosystems will need to adapt themselves to these changes or perish (Sentis et al. 2015). How effective animals are at making such adaptations is therefore interesting for predicting how global warming affects biodiversity in the future. In the extreme case we could ask whether we will see adapted species or whether the ecosystems will simply die out (Sentis et al. 2015, Quintero and Wiens 2013)?

It is however a very complex task to make predictions about the changes in the environment and how these changes affect the organisms inhabiting a certain geographical area. The difficulty lies in understanding how environmental and biotic variables interact in the natural world, and taking all of them into account is almost impossible. Sentis and colleagues (2015) argue that plasticity will be more important than evolutionary change when it comes to species survival in changing ecosystems. They argue that it is a very complex task to determine how an ecosystem will be affected by global warming, due to the complex and often poorly understood interactions between species. A good start is therefore to look at environmental factors and species one by one with a focus on phenotypic plasticity.
Garten and Gentry (1976) performed an experiment using dragonfly larvae from heated streams, examining temperature adaption. They utilized the concept of Critical thermal maximum (CTMax) (Cowles and Bogert 1944): the temperature at which the dragonflies could no longer perform. The authors also define the lethal temperature (LT) as the point when the larvae stopped all bodily movements (e.g. when they died). Their experiment showed that species living in standing waters were better at acclimating to their thermal environment compared to species in running waters, and they found a correlation between body length and greater tolerance to thermal changes (Garten and Gentry 1976). The authors suggest that this can be attributed to body size; a larger body size is better for surviving changes in temperature.

Species seem to differ in their degree of thermal adaptation with regard to habitat use (Garten and Gentry 1976, Angilletta et al. 2010, Ward and Stanford 1982, Dallas and Rivers-Moore 2012), and many aquatic organisms differ in their use of water depth. For example, Johansson (2000) showed that dragonfly species differ in their habitat use, some live close to the bottom and others close to the surface. However, we have little knowledge on if and how these species differ in their thermal adaptation. Since those living close to the bottom do not experience large temperature differences, they should be less efficient in their acclimation compared to species living close to the surface. Such difference in temperature might be around 4 to 25 degrees. This is supported by Houghton and Shoup (2013) who came to this conclusion when they examined the temperature tolerance in dragonflies. They noted that dragonfly larvae that were living in cold-water environments were more susceptible to changes in temperature. If organisms differ in thermal adaptation they would be differently affected by climate change. For example, species at the surface would be affected first by the fluctuating thermal changes, since water temperature should fluctuate more at the surface compared to the bottom. Unless they can adapt they will likely suffer more. Those at the bottom should be less adapted for changes, but thanks to the heat capacity of water they should not be affected right away, but rather later when the increase in temperature heats the whole lake and the bottom becomes uninhabitable for them, they might suffer due to inability to adapt.

The purpose of my project was to study the ability of dragonfly (Odonata) larvae to acclimate to changes in water temperature using a phenotypic plasticity approach. Dragonflies and damselflies have been used before as a model system for various experiments in the field of thermal biology (e.g. Garten and Gentry 1976, Martin et al. 1976, Nilsson-Örtman et al.)
2012). They have also been used in research related to the effects of climate change and rising temperatures (Mccauley et al. 2015, Suhling et al. 2015) and to habitat loss (Hassall et al. 2007, Hassall and Thompson 2008, Bush et al. 2014). They are an interesting group to study because they are common in aquatic ecosystems, and in addition they are predators both in water (as larvae) and as adults on land. This gives them a unique interaction on several trophic levels in the surrounding ecosystem.

In this study, which consists of two parts, I used damselflies and dragonflies that are living in Swedish lakes and ponds. First I investigated depth use in damselflies and dragonflies by performing a field study where I sampled larvae from different depths in three lakes. In the second part I studied acclimation to temperature changes in three different species of damselflies. By raising the larvae at one temperature and studying adaption at two different temperatures, acclimation could be estimated, see Figure 1.

My prediction was that there would be a clear difference in species distribution along a depth gradient in standing waters. I also predicted that species living close to the bottom would be less efficient in their temperature adaptation due to low changes in water temperature over the season. The thermal performance would change less for species that experience fluctuating temperatures, as they are already adapted to a changing environment (Angert et al. 2011, Janzen 1967).

![Figure 1. Hypothetical acclimation curve. The curves show how the performance of a trait for a specific temperature changes over time. The middle curve (B) is the temperature the organism has been exposed to prior to estimation of performance: it is the baseline. The upper curve (A) is exposure to a higher temperature, and the lower (C) is exposure to a lower temperature.](image-url)
temperature. Note how the upper and lower curves converge as time goes on; this is suggesting acclimation in performance for the organism.

**Materials and Methods**

The purpose of this project was to determine water depth used by larval species of the order Odonata and to estimate acclimation in terms of prey capture behaviour in three species of damselflies.

To determine the water depth use of damselfly and dragonfly larvae, I sampled larvae in three ponds in Uppsala:

- The SLU pond: 59°48'48.8"N 17°39'56.6"E,
- Stordammen: 59°48'17.5"N 17°42'51.4"E,
- Berthåga: 59°51'37.8"N 17°34'31.7"E

The sampling took place at the following dates SLU (The Swedish agricultural university): 2016-09-06, Stordammen: 2016-09-07, Berthåga 2016-09-08 and 2016-09-22. Larvae were collected by sweeping a net back and forth three times along a 1 meter transect. This was done along the surface (0-10 cm) and the bottom (50 cm) of the waters sampled. The net was a metal kitchen strainer attached to a stick and had diameter of 20 cm and a mesh size of 1.5 mm. The dragonflies were sorted and stored in 80% alcohol in the field. Identification to species level was carried out in the laboratory using standard entomological keys on dragonflies and damselflies. Since very few larvae were sampled, the depth usage by the species was interpreted visually from graphs showing mean number of larvae at each depth and water sampled.

I compared acclimation in prey capture behaviour in three species of damselflies (Zygoptera): *Ischnura elegans*, *Erythromma najas*, *Coenagrion pulchellum*. To receive larvae for these behavioural assays I collected eggs from females in the field. The localities where:

- Kungsängen (59°50'34.9"N 17°39'59.7" E, collected June 5-2016)
- Brillinge (59°53'10.4"N 17°41'25.0"E, collected July 7-2016)
- SLU (59°48'48.8"N 17°39'56.6"E, collected July 8-2016).
Eggs were obtained from females by placing a captured female in a glass jar with moist filter paper attached to the side of the jar. Females oviposited eggs into the filter paper within 48 h and thereafter these eggs were put into small plastic containers filled with ~0.5 liters of tap water.

After hatching, the damselfly larvae were transferred to larger plastic tubs (12.5 hight x 33cm diameter) filled with ~4 litres of water. The larvae were distributed over three plastic tubs for each species, and larvae were from mixed females of the same species. I also added some grass stems to these tubs. Adding grass facilitates bacterial growth that benefits the survival of the larvae (unpublished results). The tubs were placed in a larger water bath that held a temperature of 21 °C and had a light level that went on and off at 06:00 and 20:00, creating a day night regime of 14:10 hours. The larvae were raised in these tubs for approximately 12 weeks for *Erythromma najas* and 16 weeks for *Ischnura elegans* and *Coenagrion pulchellum*. The difference in rearing time was due to the eggs taking different amount of time to hatch and due to collection of the eggs on different dates. Also, the larvae needed different amount of time to grow to an appropriate size before experiment could start. The water in the tubs was oxygenated continuously with air from a pump. After the 12 or 16 weeks of growth and development of the larvae, acclimation with regard to prey capture behaviour was estimated at 18 and 24 °C. In addition, prey capture behaviour was measured at the baseline of 21 °C.

During the growth and development, the damselfly larvae in the tubs were fed daily with brine shrimps (*Artemia salina*). The brine shrimps where reared according to conditions provided by supplier. For more on hatching of the *Artemia* cysts see Sorgeloos et al. (1977).

I measured acclimation in two prey capture behaviours: number of strikes against prey and capture success on these. Prey capture behaviour was measured at 18 °C, 21 °C and 24 °C, where 21 °C corresponded to the baseline temperature against which acclimation was compared. Number of strikes and capture success was estimated after 1, 2, 12 and 24 hours of acclimation for *E. najas* and 1 and 12 hours for *I. elegans* and *C. pulchellum* (due to time constraints). For this, larvae were transferred from the rearing tubs and were thereafter allowed to acclimate at the three temperatures for the time frame given above, after which the two prey capture behaviours were estimated. The measurement of prey capture behaviour was done as follows: from the *Artemia* rearing containers I extracted a volume of 1ml with ca 100 *Artemia*. This solution was added to the testing containers with the damselfly larvae. The testing container had a diameter of 11 cm and was filled with ~600 ml water, which
corresponds to a water depth of ~8 cm. During the course of 10 minutes I observed the number of strikes against the prey (Artemia), and how many food items a larva was able to catch and consume (capture success). Capture success was calculated as number of successful strikes/total number of strikes. After these behavioural observations were finished, larvae were photographed in a Petri dish against a background of a millimetre paper. The lengths of the larvae were thereafter estimated in the software imageJ (ImageJ National Institute of Health, USA version 1.51h. For more info see Schneider et al. 2012).

The measurement of size I used was the distance between the outer margins of the eyes of the larvae. This measurement is proportional to body length (Corbert 1999). The reason why body length was not measured directly is because the dragonflies can contract and extend the abdomen. Measuring the size directly by length would therefore give a measurement of some uncertainty.

To take into account larval size effect, for example, that larger specimens would be better at acclimating, and time effects, for example physiology changes during ontogeny, I used as similar as possible larval size (my judgement) and run all experiment in a mixed order with regard to temperature. Occasionally I run out of untested larvae in the experiment. When that happened, I randomly choose larvae among those already tested. About 20 individuals were used twice.

Since species and larvae still differed in size, all prey capture behaviours were size corrected by using a linear model with size as the independent variable and prey capture behaviour as the dependent variable. The residuals from these two analyses were saved and used as an estimate of number of strikes and capture success respectively in my analyses.

I first ran all experiments on Erythromma najas (all temperatures and times). Due to time constraints, it was not possible to run all time periods for the other two species. After consulting the preliminary data, I concluded that for these species the time 1 h and 12 h would capture the desired intervals when acclimatization takes place, and therefore no estimates on prey capture behaviour were made for Ischnura elegans and Coenagrion pulchellum at 2 and 24 hours of acclimation (Table 1).
Table 1 Overview of species that were tested for temperatures and times of acclimation.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Time</th>
<th>1 hour</th>
<th>2 hour</th>
<th>12 hour</th>
<th>24 hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>18°C</td>
<td>Erythromma najas</td>
<td>E. najas</td>
<td>E. najas</td>
<td>E. najas</td>
<td>E. najas</td>
</tr>
<tr>
<td></td>
<td>Ischnura elegans</td>
<td>I. elegans</td>
<td>I. elegans</td>
<td>I. elegans</td>
<td>I. elegans</td>
</tr>
<tr>
<td></td>
<td>Coenagrion pulchellum</td>
<td>C. pulchellum</td>
<td>C. pulchellum</td>
<td>C. pulchellum</td>
<td>C. pulchellum</td>
</tr>
<tr>
<td>21°C</td>
<td>Erythromma najas</td>
<td>E. najas</td>
<td>E. najas</td>
<td>E. najas</td>
<td>E. najas</td>
</tr>
<tr>
<td></td>
<td>Ischnura elegans</td>
<td>I. elegans</td>
<td>I. elegans</td>
<td>I. elegans</td>
<td>I. elegans</td>
</tr>
<tr>
<td></td>
<td>Coenagrion pulchellum</td>
<td>C. pulchellum</td>
<td>C. pulchellum</td>
<td>C. pulchellum</td>
<td>C. pulchellum</td>
</tr>
<tr>
<td>24°C</td>
<td>Erythromma najas</td>
<td>E. najas</td>
<td>E. najas</td>
<td>E. najas</td>
<td>E. najas</td>
</tr>
<tr>
<td></td>
<td>Ischnura elegans</td>
<td>I. elegans</td>
<td>I. elegans</td>
<td>I. elegans</td>
<td>I. elegans</td>
</tr>
<tr>
<td></td>
<td>Coenagrion pulchellum</td>
<td>C. pulchellum</td>
<td>C. pulchellum</td>
<td>C. pulchellum</td>
<td>C. pulchellum</td>
</tr>
</tbody>
</table>

Prey capture behaviour at the acclimation temperature and times were compared between species with ANOVAs. I first performed a three way ANOVA on strikes against prey and capture success using, time temperature and species as factors. These three way ANOVAs provided information on whether species differed in acclimation. Thereafter I performed two way ANOVAs on strikes against prey and capture success using time and temperature as factors. These ANOVAS provided detailed information within species, with regard to acclimation. All statistical analyses were performed in R (version 3.2.2) (R core team 2015) and all graphs were made in excel (Microsoft excel 2007).
Results

Species depth distribution

The species found in Stordammen and their depth distribution is shown in Figure 2. The majority of the species: *Coenagrion pulchellum*, *Aeshna grandis*, *Somatochlora arctica*, *Coenagrion armatum*, *Libellula quadrimaculata* were found at the surface. In contrast *Cordulia aenea* was found only at the bottom. *Coenagrion hastulatum* was mostly found close to the surface, but unlike the others it was also found at the bottom.

![Figure 2](image.png)

**Figure 2.** The depth distribution of species found in Stordammen 2016-09-07. Bars represent total number of individuals sampled.
At the SLU pond *Coenagrion pulchellum* and *Coenagrion hastulatum* showed their highest abundance at the surface but they were also found at the bottom, though few in numbers (Figure 3). *Erythromma najas* was primarily found in the deep part of the pond with some individuals found at the surface. The other 3 species (*A. grandis, I.elegans and C. lunulatum*) occurred in very low numbers (Figure 3).

**Figure 3.** The depth distribution for species found at the SLU pond 2016-09-06. Bars represent total number of individuals sampled.
The depth distribution of species varied at the first date of sampling (2016-09-08) in the Berthåga pond (Figure 4). Both Coenagrion pulchellum and Coenagrion hastulatum were found at the deep part and at the surface, and when it comes to Coenagrion hastulatum the only difference between bottom and surface was one individual (Figure 4). Erythromma najas, Aeshna grandis and Aeshna juncea were only found at the surface. Aeshna cyanea was only found at the bottom of this pond.

During the second sampling period in the Berthåga pond (Figure 5), the data received showed a slightly different depth distribution compared to the previous time the pond was sampled. All species had the majority of their depth distribution at the surface (Figure 5). The only species found in the deep parts of the pond was Coenagrion hastulatum and Cordulia aenea.

In summary, the majority of the species were found at both the surface and bottom of the ponds, but they were in general more abundant at the surface. A few species had a higher abundance at the bottom and these were: C. aenea, A. grandis and A. cyanea
**Figure 5.** Depth distribution of species found in the Berthåga pond 2016-09-22. Bars represent total number of individuals sampled.

**Acclimation – Strikes**

*Comparison among species*

To compare if species differed in their acclimation on number of strikes, I performed a three way ANOVA. This ANOVA showed a non-significant three-way interaction between time, temperature and species and therefore I ran another ANOVA without this interaction. The subsequent ANOVA without this three-way interaction showed a significant species effect, and significant effects of the interactions between temperature and species and between time and temperature (Table 2). This suggests that species showed temperature acclimation but that this acclimation differs between them (Figure 6A-C).
Table 2. Three-way ANOVA results for *Erythromma najas*, *Ischnura elegans* and *Coenagrion pulchellum*. All temperatures, but only the times 1h and 12h were included. Strikes was the response variable.

<table>
<thead>
<tr>
<th>Response: strikes</th>
<th>Df</th>
<th>Mean sq</th>
<th>F-value</th>
<th>P-Value</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>1</td>
<td>166.39</td>
<td>1.0883</td>
<td>0.29782</td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>1</td>
<td>108.68</td>
<td>0.7108</td>
<td>0.39994</td>
<td></td>
</tr>
<tr>
<td>Species</td>
<td>2</td>
<td>3060.23</td>
<td>20.0157</td>
<td>8.22*10^-9</td>
<td></td>
</tr>
<tr>
<td>Time:species</td>
<td>2</td>
<td>145.53</td>
<td>0.9518</td>
<td>0.38737</td>
<td></td>
</tr>
<tr>
<td>Temperature:species</td>
<td>2</td>
<td>555.68</td>
<td>3.6345</td>
<td>0.02775</td>
<td></td>
</tr>
<tr>
<td>Time:temperature</td>
<td>1</td>
<td>972.59</td>
<td>6.3613</td>
<td>0.01226</td>
<td></td>
</tr>
</tbody>
</table>
Figure 6: Acclimation curves showing residual values for strikes for A) *Erythromma najas* at 1, 2, 12, 24 hours, for B) *Ischnura elegans* at 1 and 12 hours and for C) *Coenagrion pulchellum* at 1 and 12 hours.
**Erythromma najas**

The 21 °C curve is at the rearing temperature and should be the baseline for number of strikes (figure 6A). At this temperature number of strikes peaked at 2 hours of acclimation, then dropped back at 12 hours to the same level of performance as at 1 hour and then finally at 24 hours the number of strikes decreased below the levels of performance at 1 hour (Figure 6A). Larvae that were exposed to a temperature of 18 °C showed a relatively low performance in terms of number of strikes, until after 12 hours of acclimation where performance went up. At 24 hours it decreased down again. At 24 °C there was a decline in number of strikes from 1 hour to 12 hours. But after 24 hours the number of strikes increased almost to the same levels as after 2 hours of acclimation. In summary, strikes were higher at high temperatures compared to at low temperatures, and there seemed to be some acclimation since number of strikes converged at 12 hours of acclimation. This interpretation was supported by a two way ANOVA that showed a significant temperature and time effect (Table 3). However the interaction between temperature and time was non-significant (Table 3).

**Table 3:** Two-way ANOVA results on number of strikes for *Erythromma najas*. All temperatures and times were included in the analysis.

<table>
<thead>
<tr>
<th>Response: strikes</th>
<th>Df</th>
<th>Mean sq</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>1</td>
<td>606.41</td>
<td>5.4891</td>
<td>0.02025</td>
</tr>
<tr>
<td>Temperature</td>
<td>1</td>
<td>2727.29</td>
<td>24.6869</td>
<td>1.589*10^-6</td>
</tr>
<tr>
<td>Time:temperature</td>
<td>1</td>
<td>110.48</td>
<td>2.2663</td>
<td>0.13401</td>
</tr>
</tbody>
</table>

**Ischnura elegans**

At 21 °C there was no change in number of strikes over time (Figure 6B). At 18 °C there was an increase in number of strikes after 12 hours, but at 24 °C there was no change in number of strikes over time (Figure 6B). However, the two-way ANOVA showed that the number of strikes observed was non-significant since neither, time, temperature or the interaction was significant (Table 4).
Table 4: Two-way ANOVA results on number of strikes for *Ischnura elegans*. All temperatures and times were included in the analysis.

<table>
<thead>
<tr>
<th>Response:strikes</th>
<th>Df</th>
<th>Mean sq</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>1</td>
<td>371.45</td>
<td>1.5083</td>
<td>0.2228</td>
</tr>
<tr>
<td>Temperature</td>
<td>1</td>
<td>0.93</td>
<td>0.0038</td>
<td>0.9513</td>
</tr>
<tr>
<td>Time:temperature</td>
<td>1</td>
<td>476.40</td>
<td>1.9344</td>
<td>0.1679</td>
</tr>
</tbody>
</table>

*Coenagrion pulchellum*

Number of strikes did not change at the baseline 21°C (Figure 5C). At 18 °C numbers of strikes dropped over time, however it did not change over time at 24 °C (Figure 5 C). It is notable that the number of strikes was lower at 24 °C. However, the two-way ANOVA showed that the differences between the temperatures observed was non-significant since neither, time, temperature or the interaction was significant (Table 5).

Table 5: Two-way ANOVA results on number of strikes for *Coenagrion pulchellum*. All temperatures and times were included in the analysis.

<table>
<thead>
<tr>
<th>Response:Strikes</th>
<th>Df</th>
<th>Mean sq</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>1</td>
<td>23.432</td>
<td>0.1906</td>
<td>0.6635</td>
</tr>
<tr>
<td>Temperature</td>
<td>1</td>
<td>213.605</td>
<td>1.7372</td>
<td>0.1910</td>
</tr>
<tr>
<td>Time:temperature</td>
<td>1</td>
<td>3.155</td>
<td>0.0257</td>
<td>0.8731</td>
</tr>
</tbody>
</table>

Capture success

Comparison among species

The three-way ANOVA on capture success showed a non-significant three-way interaction between time, temperature and species and therefore this interaction was removed. The subsequent ANOVA with this three-way interaction removed showed a significant species effect, and significant effect of the interaction between time and species, suggesting that species differ in capture success and that this difference changes over time (Table 6, Figure 7A-C).
Table 6: Three-way ANOVA results on capture success on prey. All temperatures, but only the time periods 1h and 12h were included. Capture success is the response variable.

<table>
<thead>
<tr>
<th>Response: capture success</th>
<th>Df</th>
<th>Mean sq</th>
<th>F-value</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>1</td>
<td>0.12145</td>
<td>1.8318</td>
<td>0.17634</td>
</tr>
<tr>
<td>Temperature</td>
<td>1</td>
<td>0.03288</td>
<td>0.4977</td>
<td>0.48116</td>
</tr>
<tr>
<td>Species</td>
<td>2</td>
<td>1.3670</td>
<td>20.6858</td>
<td>4.605*10^-9</td>
</tr>
<tr>
<td>Time:species</td>
<td>2</td>
<td>0.23314</td>
<td>3.5288</td>
<td>0.03075</td>
</tr>
<tr>
<td>Temperature:species</td>
<td>2</td>
<td>0.0684</td>
<td>0.0694</td>
<td>0.93300</td>
</tr>
<tr>
<td>Time:temperature</td>
<td>1</td>
<td>0.9603</td>
<td>0.9603</td>
<td>0.32803</td>
</tr>
</tbody>
</table>
Figure 7: Acclimation curves showing residual values for capture success for A: *Erythromma najas* at 1, 2, 12, 24 hours, for B: *Ischnura elegans* at 1 and 12 hours, and for C: *Coenagrion pulchellum* at 1 and 12 hours.

**Erythromma najas**

At 21 °C the capture success for *E. najas* showed a decline towards 12 hours, after which it increased somewhat again (Figure 7A). The 18 °C curve showed a sharp decline in capture success after 2 hours, and after 12 hours the capture success went up again, but it never reached the level that was observed at 1 hour (Figure 7A). Finally, the capture success went
down again at 24 hours, but only to change again, approximately halfway to the performance of 2 hours of acclimatization. At 24 °C there was a slight decline in capture success between 1 hour and 2 hours, but thereafter it went up again at 12 and 24 hours (Figure 7A). A two-way ANOVA showed however, that these changes were non-significant since neither, time, temperature or interaction was significant (Table 7).

**Table 7:** Two-way ANOVA results on capture success for *Erythromma najas*.
All temperatures and times were included in the analysis

<table>
<thead>
<tr>
<th>Response: capture success</th>
<th>Df</th>
<th>Mean sq</th>
<th>F value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>1</td>
<td>0.000420</td>
<td>0.0058</td>
<td>0.9395</td>
</tr>
<tr>
<td>Temperature</td>
<td>1</td>
<td>0.080344</td>
<td>1.1060</td>
<td>0.2944</td>
</tr>
<tr>
<td>Time:temperature</td>
<td>1</td>
<td>0.052588</td>
<td>0.7239</td>
<td>0.3960</td>
</tr>
</tbody>
</table>

For *I. elegans* all the curves for capture success at 18, 21 and 24 °C showed an increase from 1 hour to 12 hours (Figure 7B). This pattern was supported by the two-way ANOVA that showed a significant effect of time, and a non-significant effect of temperature and the interaction between temperature and time (Table 8).

**Table 8:** Two-way ANOVA results on capture success for *Ischnura elegans*. All temperatures and times were included in the analysis.

<table>
<thead>
<tr>
<th>Response: capture success</th>
<th>Df</th>
<th>Mean sq</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>1</td>
<td>0.57269</td>
<td>8.1291</td>
<td>0.005453</td>
</tr>
<tr>
<td>Temperature</td>
<td>1</td>
<td>0.00112</td>
<td>0.0159</td>
<td>0.899985</td>
</tr>
<tr>
<td>Time:temperature</td>
<td>1</td>
<td>0.0750</td>
<td>0.0750</td>
<td>0.784788</td>
</tr>
</tbody>
</table>

The capture success of *Coenagrion pulchelum* showed a convergence after 12 hours to the same number of captures, and hence at 18 °C there was an increase and at 24 °C there was a decrease (Figure 7C). The results from the ANOVA showed however non-significant effect of time, temperature and the interaction (Table 9).
Table 9: Two-way ANOVA results on capture success for Coenagrion pulchellum. All temperatures and times were included in the analysis.

<table>
<thead>
<tr>
<th>Response: Capture success</th>
<th>Df</th>
<th>Mean sq</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>1</td>
<td>23.432</td>
<td>0.0143</td>
<td>0.9051</td>
</tr>
<tr>
<td>Temperature</td>
<td>1</td>
<td>213.605</td>
<td>0.1407</td>
<td>0.7085</td>
</tr>
<tr>
<td>Time:temperature</td>
<td>1</td>
<td>3155</td>
<td>0.2300</td>
<td>0.6327</td>
</tr>
</tbody>
</table>
Discussion

My results show that species had different performances at different temperatures and that there was a difference between species in how well they performed. This is important as it tells us that under the scenario of increased temperature in the lakes, species will have different larval growth and development which ultimately could affect the adult stage through mating success and egg production. In the long run, this might have an impact on competition between these species and might result in some species going extinct in local ponds. Obviously it will take some generations for a species of Odonata to completely go extinct, especially, as they have the advantage of dispersing quite far from their emerging water. Hence, maybe we will see a difference in where the dragonflies distribute themselves and probably there will be a change in the mosaic distribution of local species.

Depth distribution by Odonata

Many damselfly and dragonfly larvae differ in their depth distribution. Wissinger (1998) showed that dragonfly larvae Perithemis tenera preferred a depth of 1.0-1.5 meters while Erythemis simplicocollis preferred shallow water at a depth of < 0.5 meter. In a laboratory experiment, Johansson (2000) showed that Lestes sponsa and Coenagrion hastulatum exploited microhabitats close to the surface, while Cordulia aenea and Leucorrhinia dubia used microhabitats close to the bottom. I also found differences in depth distribution in the waters I sampled. Looking at the depth distribution (figure 2-5) it is clear that some species are more common than others, and the Coenagrionidae damselflies (e.g. Coenagrion pulchellum/puella, Coenagrion hastulatum and Erythromma najas) are the ones I sampled the most.

The distribution of these species varied between localities, but both C. pulchellum/puella and C. hastulatum were mostly found in the upper part of the water, while E. najas was found in the deep part in one locality (Figure 2) and in the upper part at Berthåga (Figure 3 and 4). I found very few I. elegans in the ponds, therefore I could not make any strong predictions with regard to acclimation in this species. However, the depth distribution suggests that C. pulchellum should be better at acclimation than E. najas since the latter was found at deeper waters in at least one of the ponds. Alternatively, based on the results from the depth distribution one could argue that C. pulchellum and E. najas should show a strong and fast
acclimation, since these two species were found both at the surface and in the depths. Thus, they should be better at adapting to changing temperatures, as they live in varied environments.

In summary, according to the depths distribution, *C. pulchellum* should be exposed to a largely variable environment as it lives in the shallow parts, and thus if the idea of large variation holds up it should be better at adapting to new temperatures. *E. najas* on the other hand was found at both the depths and the shallow parts which could also be the basis of pre-adaptation for surviving fluctuations in temperature better. One could also make the argument that both environments fluctuate but with different intensities and amplitude. In this case the rate at which acclimating occur, would matter for the ability to survive in the larvae.

Logically, the species living at the bottom of the lakes should be less adaptable to thermal change since this environment is more thermally stable during the day, in comparison to the ones living at the surface whom will have a more variable environment daily and thus need to be more plastic and adaptable to new temperatures. Unfortunately, none of the species that have their main distribution close to the bottom were part of my laboratory experiments on acclimation.

*Acclimation – laboratory experiment*

My results on strikes and capture success towards prey show that species differ in strikes, with *E. najas* having the highest number of strikes and *C. pulchellum* the lowest. The results also suggest that the performance for the larvae changes over time and that this change over time differs between species since the three way ANOVA showed significant interaction terms with regard to temperature:species and time:species. A similar result was found for capture success, except that the interaction temperature:species was non-significant, suggesting that species changed in the same way over time in capture success.

Looking at the graphs and ANOVA for each separate species, *E.najas* has the most data-points and therefore allows for a thorough comparison among temperatures. There is a tendency for acclimation in strikes and capture success since the 18 C° and 24 C° curves convergence towards the 21 C° rate. However, the interaction term between time and temperature was non-significant. One reason for the non-significant results is the large variance in the dataset. Both *C. pulchellum* and *I. elegans* have tendencies for the same
direction in all the changes in prey capture behaviour over time, however no evidence for acclimation was found.

Very few studies have been performed on prey capture behaviour and acclimation in damselflies. Thompson (1978) found that *Ischnura elegans* prey capture did not increase at temperature above 20-27 °C, and argued that 27 °C might lay outside the range the larvae would experience in the field even at shallow waters in the summer. This indicates that the performance at 24 °C for *I. elegans* might be at the maximum temperature where the species can optimally hunt. Thus acclimatisation to higher temperatures might be difficult for this species, even though it lives close to the surface. Garten and Gentry (1976) and Martin et al. (1976) examined temperature tolerance in dragonfly larvae from streams, and used time spent in a new temperature, as measurement of acclimation. The time the larvae were allowed to stay in the new environment was 48 hours, and was deemed sufficient for the larvae to acclimate properly. These authors also found that thermal tolerance was significantly correlated to acclimation temperature in the studied species. This suggests that larvae adapt quickly to new temperature ranges and should thus survive high temperatures in streams better.

Calosi et al. (2008) looked at the thermal limits, acclimatisation and the effects of climate change in diving beetles. They found that the beetles with the highest thermal maximum were the ones who were best at acclimating to higher temperatures. They also found that species with the lowest ability to cope with low temperatures were the ones that had the lowest ability to acclimate to high temperature. While beetles are not closely related to damselflies, if this pattern is present in the species I have studied, it could mean that dragonflies living at the surface would be better at acclimating than bottom dwellers.

In general it is assumed that species living in variable environments should be better at acclimation (Angiletta 2009). In a model, Gabriel et al. (2005) showed that seasonal acclimatization is advantageous for organisms that live in an environment that change predictably over the season, but also that habitats varying randomly would favour organisms with an intermediate ability to adapt. This suggests that animals that experience large seasonal variance should have the largest capacity for acclimatization due to large variation in temperature. Schaefer and Ryan (2006) tested this prediction in zebrafish (*Danio rerio*). They used two stabile thermal regimes, two constant diel cycles and one stochastic temperature regime. The acclimatisation in temperature tolerance varied between the different treatments,
and the fish living in a variable environment seemed to have a greater tolerance to changes than those in a stable environment, hence a support for the model by Gabriel et al. (2005). In addition, Feldmeth et al. (1974) tested pupfish adapted to high temperatures and low temperature variation and concluded that animals subjected to altering temperatures had a wider range of thermal tolerance, than those subjected to only one temperature range. These findings also support the Gabriel et al. (2005) model.

Interestingly, not all studies have found that a greater environmental variability is correlated with greater acclimation. For example, Niehaus et al. (2011) showed that the performance of tadpoles of the striped marsh frog (Limnodynastes peronei) did not differ between treatments in fluctuating and stable thermal environments over broad temperature ranges (oxygen consumption and heart rate). This suggests that the tadpoles had a limited plastic response in metabolism due to the fluctuating environment in terms of temperature, at least as larvae. The authors suggested that organisms in unstable environments decrease their thermal performance, instead of developing a wide performance breath. They also argue that maybe the increased temperature forces the animal to increase its metabolism in response to the environment, and that in shortage of energy to metabolise the animal instead catabolises its own tissues to provide energy for increased high metabolic rate. This could perhaps be the case for some of the dragonfly and damselfly larvae as well.

In an aquatic system like the ones I have studied, spring and autumn turnover changes the temperature drastically over the season, while during summer the temperatures are less variable. The former variation occurs with predictable intervals and thus we would expect that most of the species in the lakes are adapted to this variation. It would therefore be interesting to examine adaption over the seasons to see if the dragonflies have different survival rates when differences in thermal fluctuation are imposed by the seasons, rather than strictly specific degrees up or down. This would be an interesting continuation and addition to the experiments I have made. Another important factor is that these damselflies have the ability to move around quite well by swimming. It would be interesting to examine if dragonflies can use this extra mobility to track local environments and move to the deep parts if the shallow areas become too hot.

**Phylogeny**

The three species used in my study are related fairly closely, since they belong to the family Coenagrionidae. *E. najas* and *C. pulchellum* also belong to the same subfamily,
Coenagrionidae, while *I. elegans* belongs to the subfamily Ischnurinae (Dijkstra and Kalkman 2012). Since they are related, I expected them to perform approximately equal in their thermal adaption, but still differ somewhat in their degree of acclimation. The results showed that only *E. najas* had a tendency to show acclimation for the traits I examined, though it was not significant. Ideally comparisons between species should use a comparative method, since related species share many similar traits as a result of descendent and species are therefore not independent (Felsenstein 1985). Unfortunately, my data set did not allow me to correct for phylogeny.

**Confounding factors**

As previously mentioned the larvae of insects are ectotherms. I have assumed that they could acclimate when exposed to a prolonged change in temperature. It is however possible that they instead change their metabolism. If this was the case, it would result in less food eaten in cold environments due to decreased metabolism and not due to a decrease in performance. Interestingly this is not what I found, since the damselfly larvae were eating similar amounts of prey more or less regardless of the temperature after 12 hours.

A potential factor that could bias my results is the activity and movement of the *Artemia*. Since *Artemia* are also ectotherms, cold temperatures will make them slower and warm temperatures will allow them to move faster. This could affect strike and capture success. However the exposure time to the temperature was only 10 minutes while it was greater or equal to one hour for the damselfly larvae. Despite this I found that strikes and capture success changed over time at the acclimation periods used for the damselflies, suggesting that potential changes in activity and movement in *Artemia* as an effect of temperature was of minor importance for the results obtained in the damselflies.

**Conclusion**

I found that species differed in their performance across temperatures. *E. najas* had the highest number of strikes and capture success and *C. pulchellum* the lowest. I also found that species changed their prey catching behaviour in response to the time they had been exposed to a novel temperature. I did however not find any strong evidence for acclimation in prey catching behaviour. In my experiment I tested temperature adaption in species one by one. However, as species do not occur as individuals in nature, I concur with Grigaltchik et al. (2012) who wrote that one must focus not on one single species, as no species lives in
complete isolation. But instead one might have to look at the whole community and its interactions with the species to be able to make correct interpretations on how climate change will affect them. My work is on the way by comparing three species, and perhaps someone could expand this by comparing more species in a competitive situation in future studies?

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R Development Core Team (2015) R: A Language and Environment for Statistical Computing. R Foundation